PB83-231472

Cell Death and Inhibition of Population Growth of Marine Unicellular Algae by Pesticides

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1983

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1. REPORT NO. EPA-600/J-82-014	2.	3. PB83-231472
4. TITLE AND SUBTITLE Cell Death and Inhibition of Pollution Growth of Marine Unicellular Algae by Pesticides (Journal Version)		5. REPORT DATE 1983 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Gerald E. Walsh		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT NO. ACBE1A/CCBE1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, FL 32561		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES
REF: Aquatic Toxicology 3:209-214 (1983)

16. ABSTRACT

The marine diatom, Skeletonema costatum, was exposed to the pesticides hexachlorocyclopentadiene, EPN, chlorpyrifos, carbophenothion, and Atrazine and examined for death of cells with Evans blue, a mortal stain. All pesticides caused death of cells, but significant mortality occurred at concentrations greater than the EC50. The insecticide, Amdro, did not kill the marine algae S. costatum, Thalassiosira pseudonana, Isochrysis galbana, Chlorella sp., or Dunaliella tertiolecta. However, Amdro was very inhibitory to algal population growth at low concentrations, i.e., EC50's at 48 hours were between 0.14 ppb for T. pseudonana and 10.3 ppb for D. tertiolecta. EC50's were lowest after 48 hours of exposure in tests conducted for 96 hours. By 96 hours after exposure, maximum growth rates of treated cultures were approximately those of control cultures. It is recommended that, because of problems associated with the fate of toxicants, algal laboratory toxicity tests be conducted for 48 to 72 hours instead of the usual 96 hours or longer.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS (This Report) Unclassified 20. SECURITY CLASS (This page) Unclassified	21. NO. OF PAGES 7 22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-82-014
JOURNAL ARTICLE

Aquatic Toxicology, 3 (1983) 209–214
Elsevier Biomedical Press

CELL DEATH AND INHIBITION OF POPULATION GROWTH OF MARINE UNICELLULAR ALGAE BY PESTICIDES*

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(Received 25 June 1982; accepted 20 September 1982)

The marine diatom, *Skeletonema costatum*, was exposed to the pesticides hexachlorocyclopentadiene, EPN, chlorpyrifos, carbophenothion, and Atrazine and examined for death of cells with Evans blue, a mortal stain. All pesticides caused death of cells, but significant mortality occurred only at concentrations greater than the EC_{50} calculated from population growth studies. The insecticide, Amdro®, did not kill the marine algae *S. costatum*, *Thalassiosira pseudonana*, *Isochrysis galbana*, *Chlorella* sp., or *Dunaliella tertiolecta*. However, Amdro was very inhibitory to algal population growth at low concentrations, i.e. EC_{50} values at 48 h were between 0.14 ppb for *T. pseudonana* and 10.3 ppb for *D. tertiolecta*. EC_{50} values were lowest after 48 h of exposure in tests conducted for 96 h. By 96 h after exposure, maximum growth rates of treated cultures were approximately those of control cultures. It is recommended that, because of problems associated with the fate of toxicants, algal laboratory toxicity tests be conducted for 48 to 72 h instead of the usual 96 h or longer.

Key words: marine algae; pesticides; bioassay; toxicity; growth rate

INTRODUCTION

In standard toxicity tests with algae, inhibition of population growth is usually estimated without regard for potential algicidal effects of the toxicants on individual cells. Payne and Hall (1979) addressed the question of algicidal and algistatic effects of toxicants and described a reculture method for detecting such effects. Reynolds et al. (1978) used the more direct method of Crippen and Perrier (1974) to evaluate the effects of chlorine-produced oxidants (CPO) on the survival of eight species of marine algae. This method uses the vital stain, neutral red, and the mortal stain, Evans blue, to identify living and dead cells, and Reynolds et al. (1978) demonstrated that CPO did not kill cells. There are no published data for marine unicellular algae with regard to direct algicidal effects of pesticides. Such effects of five pesticides on the marine diatom, *Skeletonema costatum*, are reported here.

* Contribution No. 430 from the Gulf Breeze Laboratory.

0166-445X/83/0000–0000/$03.00 © Elsevier Science Publishers

Effects of the amidinohydrazone insecticide, Amdro®, * (American Cyanamide Co., Princeton, NJ), which is used in the southeastern United States to kill the imported fire ant, *Saevissima richteri*, on five species of unicellular algae are also reported. The manufacturer's Technical Information Sheet states that the insecticide degrades rapidly in sunlight, with a half-life of <24 h in the field. Algal toxicity tests are usually conducted under intense light for a minimum of 96 h. It was hypothesized that this time span could be too long to allow detection of toxicity. Therefore, in addition to algicidal effects, toxicity of Amdro to population growth after 48, 72, and 96 h was estimated.

MATERIALS AND METHODS

Algae were exposed to pesticides in two ways. In the first, *S. costatum* (Greville) Cleve (Chrysophyta) was exposed to the chlorinated hydrocarbon insecticide hexachlorocyclopentadiene (Hooker Chemical Co., Niagara Falls, NY), the organophosphate insecticides EPN (E.I. DuPont de Nemours and Co., Wilmington, DE), chlorpyrifos (Dursban®, Dow Chemical Co., Midland, MI) and carbophenothion (Trithion®, Stauffer Chemical Co., Mountain View, CA), and the triazine herbicide Atrazine (Ciba-Geigy Corp., Greensboro, NC). The alga was cultured and tested in sterile artificial sea water prepared from a commercial salt mix (Rila Products, Teaneck, NJ) fortified with trace metals, vitamins, and minor salts (Walsh and Alexander, 1980). Salinity was 30‰ and pH was 8.1. Initial cell concentration was 4×10^4 cells $\cdot$ ml^{-1}. Algae were grown for 48 h in 25 ml of medium in sterile test tubes (20×200 mm) capped with stainless-steel closures at 20 ± 0.5°C under 150 μE $\cdot$ m$^{-2}\cdot$ s^{-1} photosynthetically active radiation from cool white fluorescent tubes. The lighting cycle was 14 h of light followed by 10 h of darkness, and cultures were incubated with shaking at approximately 60 excursions $\cdot$ min^{-1} on Gyratory shakers (New Brunswick Scientific Co., New Brunswick, CN).

After incubation for 48 h, algal cells in all tubes were enumerated on a hemocytometer and dead cells identified by the Evans blue method of Crippen and Perrier (1974) in order to determine the percentage of dead cells in normal cultures. Pesticide was added in 0.1-ml nanograde acetone and five concentrations were used, the highest being approximately the saturating concentration in sea water of 30‰ salinity. Each concentration and the acetone-treated controls were replicated three times. The algae were incubated after treatment for 48 h and living and dead cells again enumerated. The concentration that would inhibit growth by 50% (EC_{50}) was estimated by straight-line graphical interpolation (APHA, 1975) and the percentage of dead cells calculated (average values of each of the three exposures).

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* Reference to trade names in this publication does not constitute endorsement by the U.S. Environmental Protection Agency.

In the second test, the chrysophytes *S. costatum, Thalassiosira pseudonana* Hasle and Hundal, and *Isochrysis galbana* Parke, and the chlorophytes *Dunaliella tertiolecta* Butcher and *Chlorella* sp. were exposed to the amidinohydrazone insecticide Amdro. Algae were cultured and exposed in the artificial sea water of Harrison et al. (1980). Salinity was 30‰, pH adjusted to 8.1, and culture conditions the same as described above, except the toxicant, in nanograde acetone, was introduced at the beginning of the test and population growth followed by cell counts after 2, 3, and 4 days of exposure. Dead cells were enumerated after staining with Evans blue. The EC_{50} and its 95% confidence interval (CI) were calculated each day by the moving average method of Stephan (1977). Growth rate (k) was calculated from the expression $k = \ln (N_t/N_0)/t$, and the maximum growth rates, k_{max}, of control and treated cultures were compared.

RESULTS

In the first test, all five pesticides caused death of cells at the higher concentrations, but to varying degrees (Table I). Numbers of dead cells in control populations and populations before treatment were low, between 0 and 0.87%. Atrazine was the least algicidal pesticide: the very high concentration of 1 mg/l (ppm) caused an average of only 1% dead cells over the average of the control populations. At the other extreme, chlorpyrifos killed 100% of the cells at 5 ppm. None of the chemicals, except chlorpyrifos at 5 ppm, caused cessation of population growth.

Population growth rate was reduced mainly by inhibition of cell division, as evidenced by depressed, but continued growth in all treated cultures except 5 ppm chlorpyrifos. Percentage of dead cells at the EC_{50} concentrations was relatively low, between approximately 0.9% for Atrazine and approximately 3.3% for carbophenothion and chlorpyrifos.

It is unlikely that any of the pesticides tested would kill appreciable numbers of algae in field exposures because of the high concentrations required, and regrowth of populations could be expected when pesticide concentrations fell below critical values.

In the second test, concentrations of Amdro as low as 0.14 μg/l (ppb) reduced growth of algae. Figure 1 shows growth of *T. pseudonana* at concentrations that bracket the EC_{50} for 4 days of population growth. Each concentration depressed growth initially, but maximum growth rates (k_{max}) after the second day of exposure were approximately the same as that of the control.

Initial strong suppression of growth, followed by recovery, was reflected in EC_{50} values calculated for 2, 3, and 4 days after exposure. Table II shows that the lowest EC_{50} value for each species occurred on the second day, except for *I. galbana*, which did not grow rapidly enough by that day to yield statistically valid data. In that case, the third-day EC_{50} was significantly lower than that of the fourth day. *T pseudonana* and *S. costatum* were the most sensitive species on each day, and the

TABLE I

Effects of pesticides on survival of *S. costatum*.

Chemical (EC_{50})[a]	Concentration	Dead (%)[b]		Chemical (EC_{50})	Concentration	Dead (%)[b]	
		Before	After			Before	After
Atrazine (265 ppb)	Control	0.56	0.83	EPN (563 ppb)	Control	0.27	0.27
	50 ppb	0.22	1.0		100 ppb	0.87	0.57
	100	0.48	1.1		250	0.20	0.53
	250	0.35	0.91		500	0.46	2.4
	500	0.83	1.3		750	0.65	18.5
	1000	0.37	1.8		1000	0.21	25.5
Hexachlorocyclo-	Control	0.83	0.21	Chlorpyrifos (1.2 ppm)	Control	0.52	0.54
pentadiene (none)	2.5 ppb	0.42	0.15		0.1 ppm	0.58	0.53
	5.0	0.56	0.19		0.5	0.71	0.96
	7.5	0.35	1.0		1.0	0.71	3.3
	10.0	0.86	1.5		2.5	0.32	88
	25.0	0.71	4.0		5.0	0.44	100
Carbophenothion	Control	0	0.15				
(352 ppb)	25 ppb	0	0.72				
	50	0.17	1.2				
	100	0.18	1.0				
	250	0.28	3.2				
	500	0.42	14.9				

[a] Concentration that inhibited growth by 50%.

[b] Before = % dead before treatment. After = % dead after 48 h of exposure.

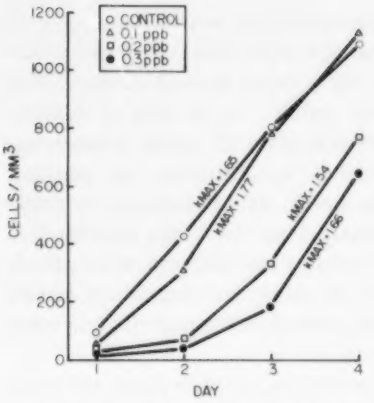

Fig. 1. Growth of *T. pseudonana* in Amdro. Each point is the average response of three cultures. k_{max} = maximum growth rate.

TABLE II

Effects of Amdro on growth of marine unicellular algae after 2, 3, and 4 days of exposure.

Species	Day	EC_{50} (ppb)[a]	95% CI
T. pseudonana	2	0.139	0.135 – 0.144
	3	0.166	0.162 – 0.170
	4	0.255	0.250 – 0.259
S. costatum	2	0.171	0.160 – 0.182
	3	0.180	0.172 – 1.188
	4	0.240	0.234 – 0.246
I. galbana	2	ND[b]	ND
	3	1.81	1.63 – 1.99
	4	2.89	2.74 – 3.06
Chlorella sp.	2	2.74	1.95 – 3.85
	3	5.49	4.76 – 6.37
	4	4.97	4.45 – 5.57
D. tertiolecta	2	10.3	7.3 – 13.4
	3	10.8	9.1 – 12.6
	4	17.8	15.6 – 20.5

[a] EC_{50} = calculated concentration that would inhibit growth by 50%.
[b] ND = population density too low for calculation of EC_{50}.

chrysophytes were significantly more sensitive than the chlorophytes.

Amro did not kill individuals of any species. Percentages of dead cells in treated cultures did not exceed those in control cultures.

Increased EC_{50} values on each day after exposure, except for *Chlorella* sp., in which the values for the third and fourth days were not statistically different, sug-

gest that physical, chemical, or biological factors that affect toxicity may occur in static algal tests with pesticides. Since the purpose of toxicity tests is to detect toxicity without consideration of fate of the toxicant, EC_{50} values derived on the second day of exposure in acute tests best approximate toxicity. In the case of Amdro, photodecomposition may have reduced the concentration of parent compound, allowing maximal growth to proceed after a critical concentration was reached. Lowest EC_{50} values are usually attained on the second day of exposure to single compounds and complex wastes (Walsh, in press). After that time, toxicant fate becomes important in assessment of toxicity. Toxicants may decompose or adsorb to the glass walls of exposure vessels. Also, after the slowly-growing culture attains a certain biomass, the ratio of biomass to toxicant concentration may rise to a value that allows exponential growth of the population.

These tests suggest that, for evaluation of effects of toxicants on algae, the cause of population growth inhibition (algicidal or algistatic) should be defined and that fate and behavior of toxicants in exposure vessels can affect toxicity. The data support the contention that long-term tests may be ineffective for detection and quantitation of toxicity. It is suggested: (1) the lowest value for expression of toxicity be considered as the value that most closely approximates true toxicity, and (2) that value may be calculated after only 2 days of exposure. If fate of the toxicant is known, then potential hazard of the toxicant to natural populations can be evaluated from mode-of-action, toxicity, and fate data.

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